

Figure 1

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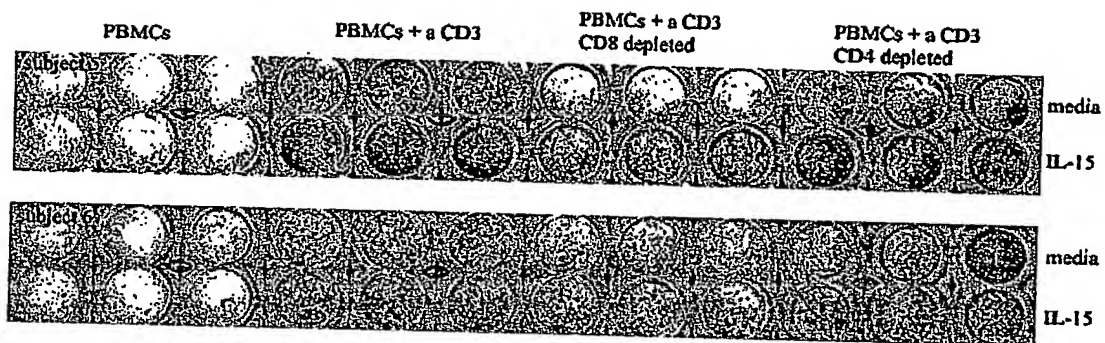


Figure 2

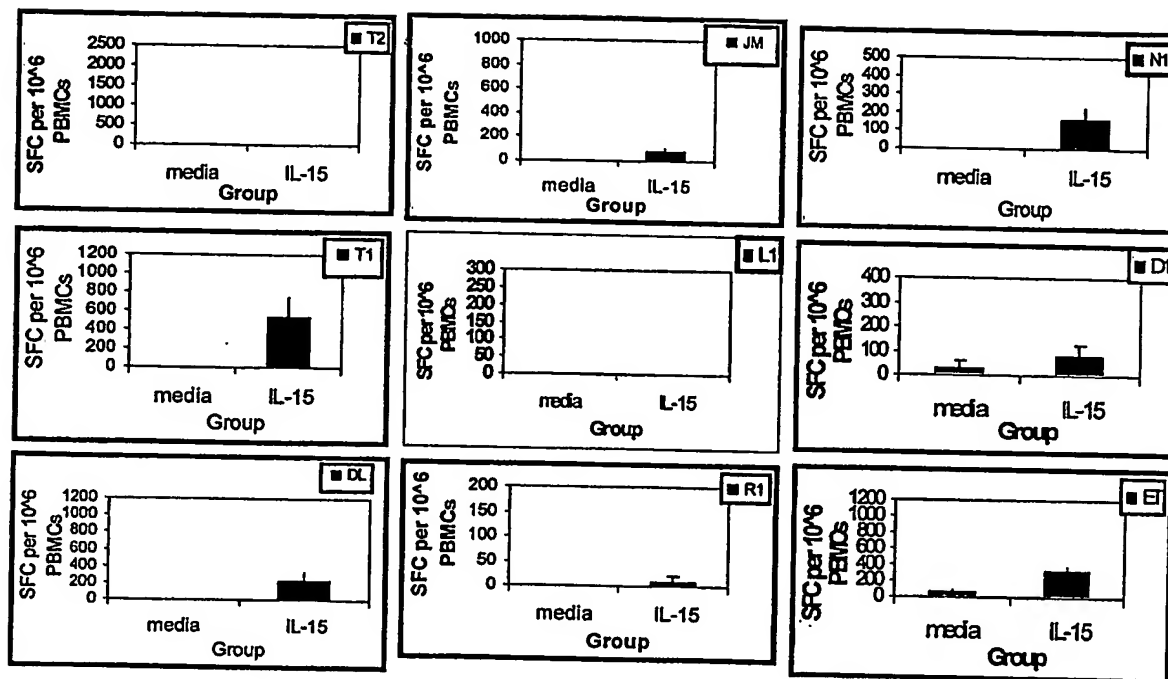


FIGURE 3A

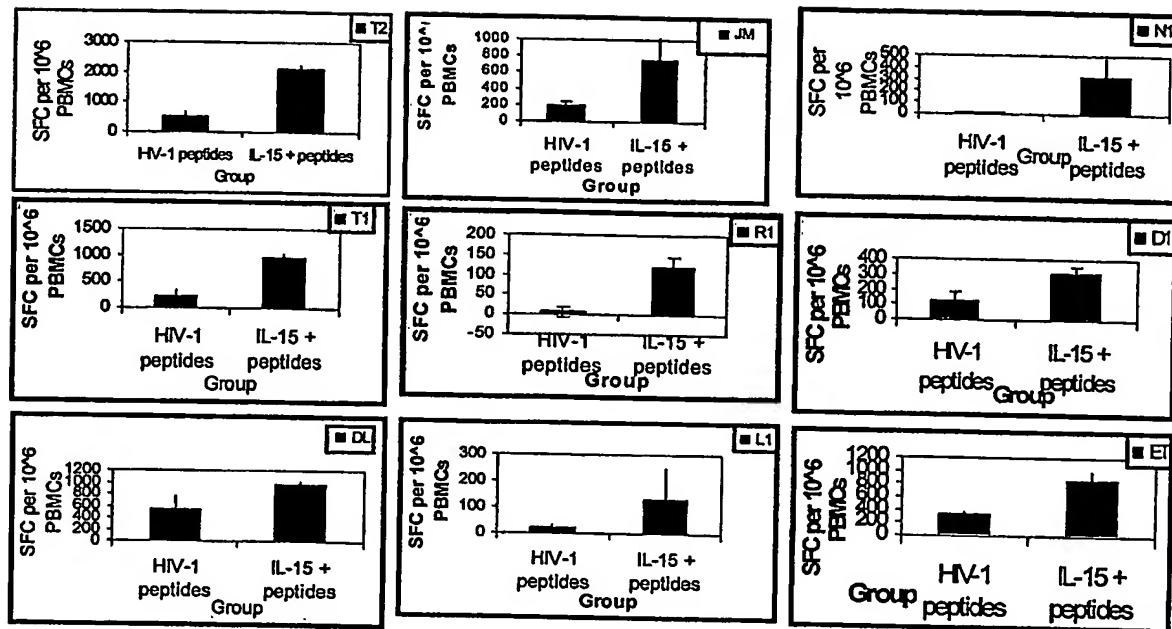


FIGURE 3B

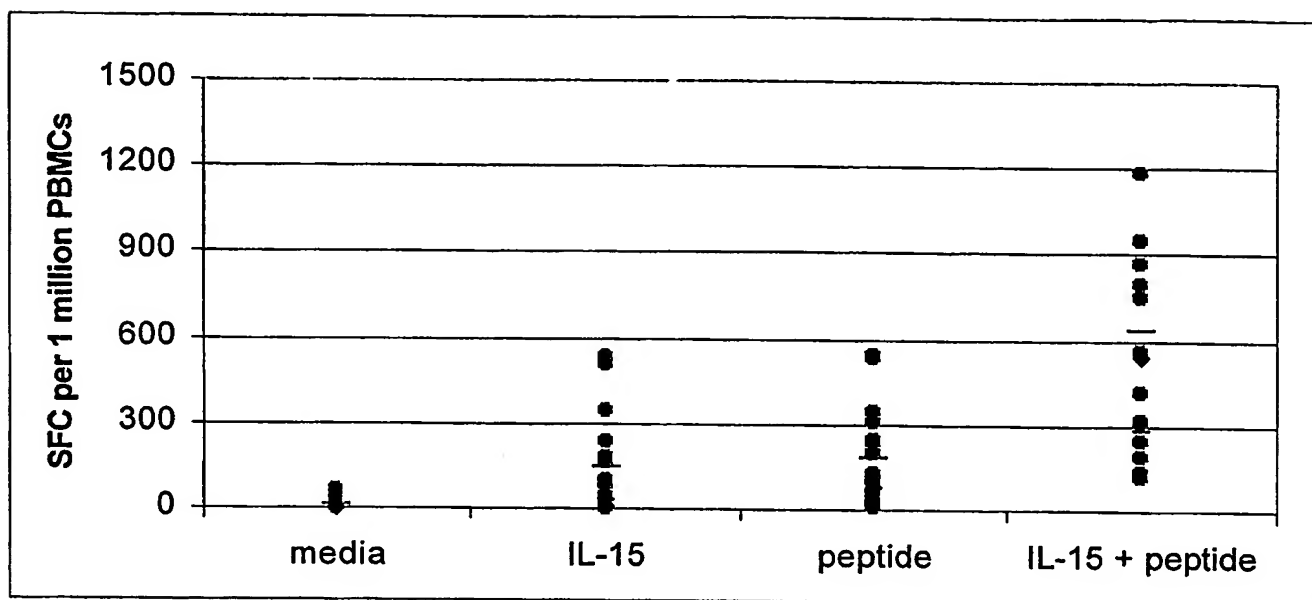


Figure 3C

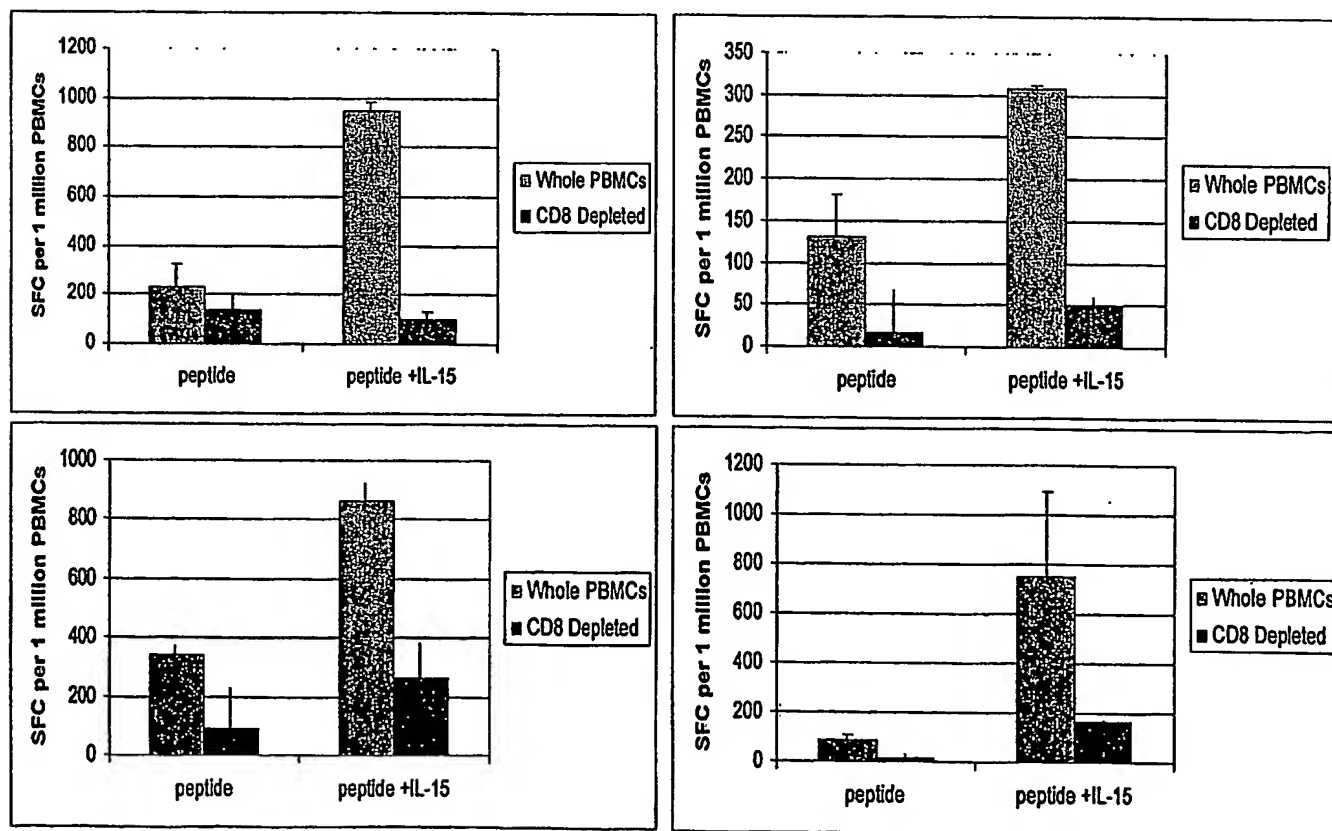


Figure 3D

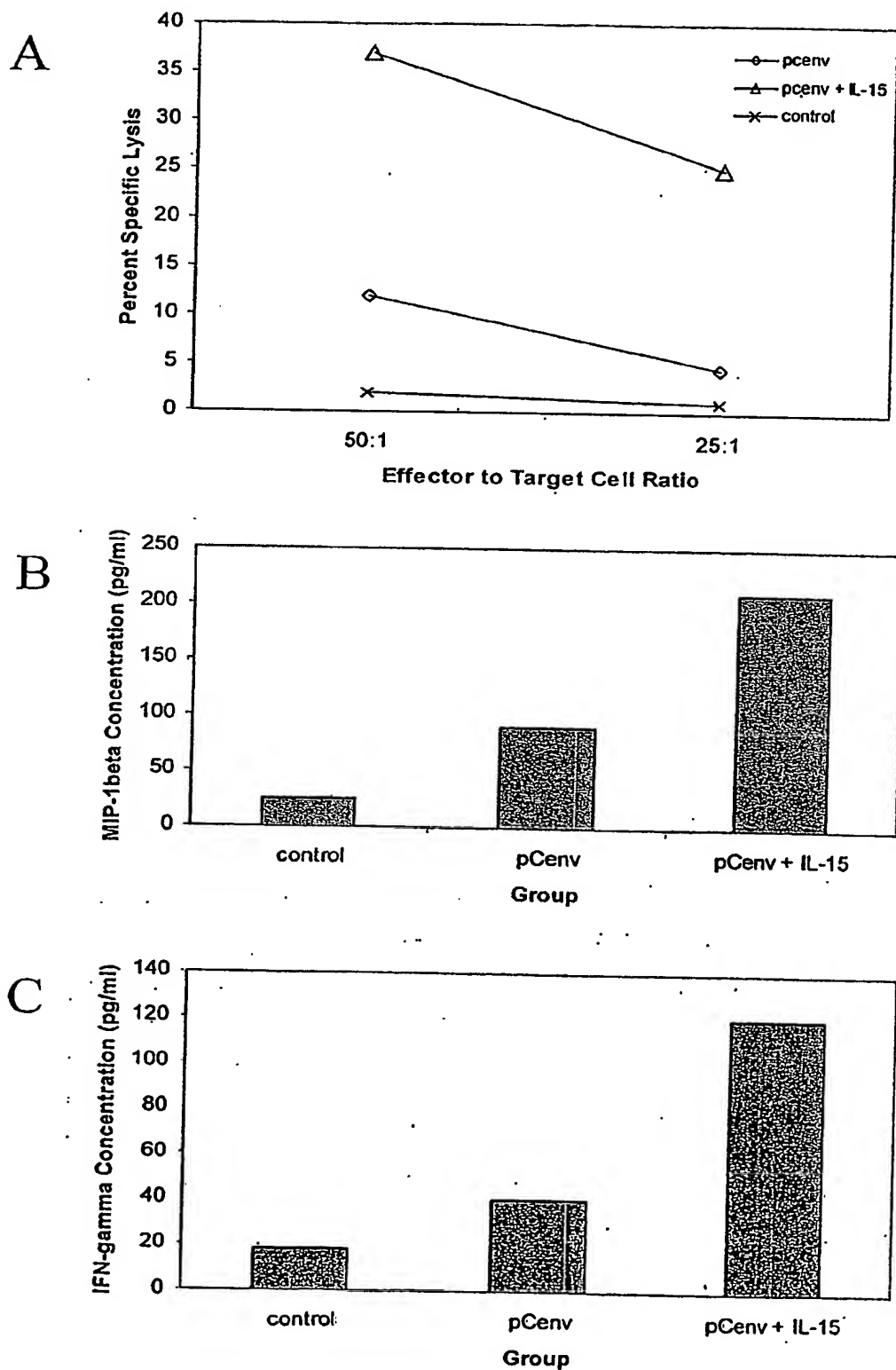


Figure 4

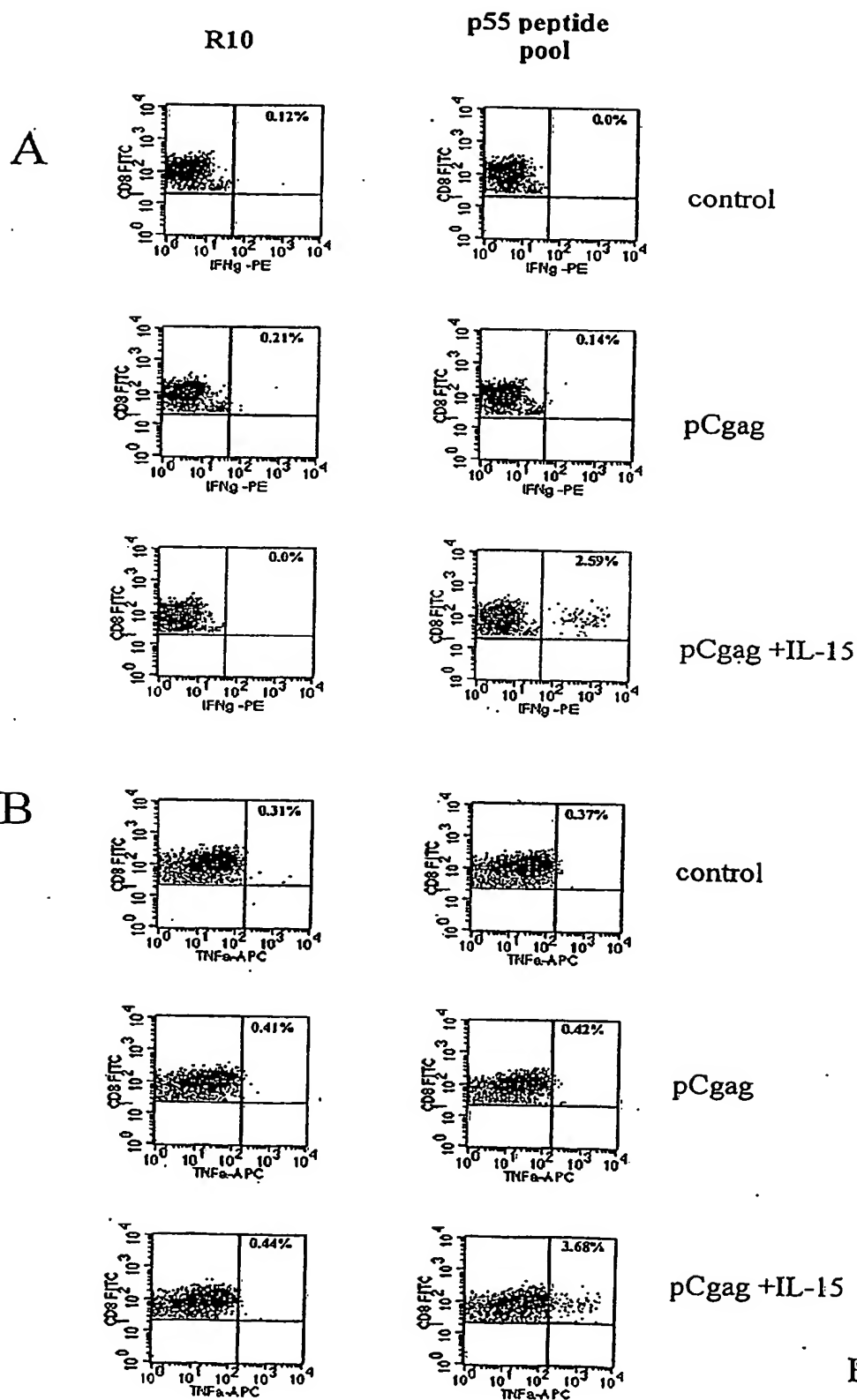


Figure 5

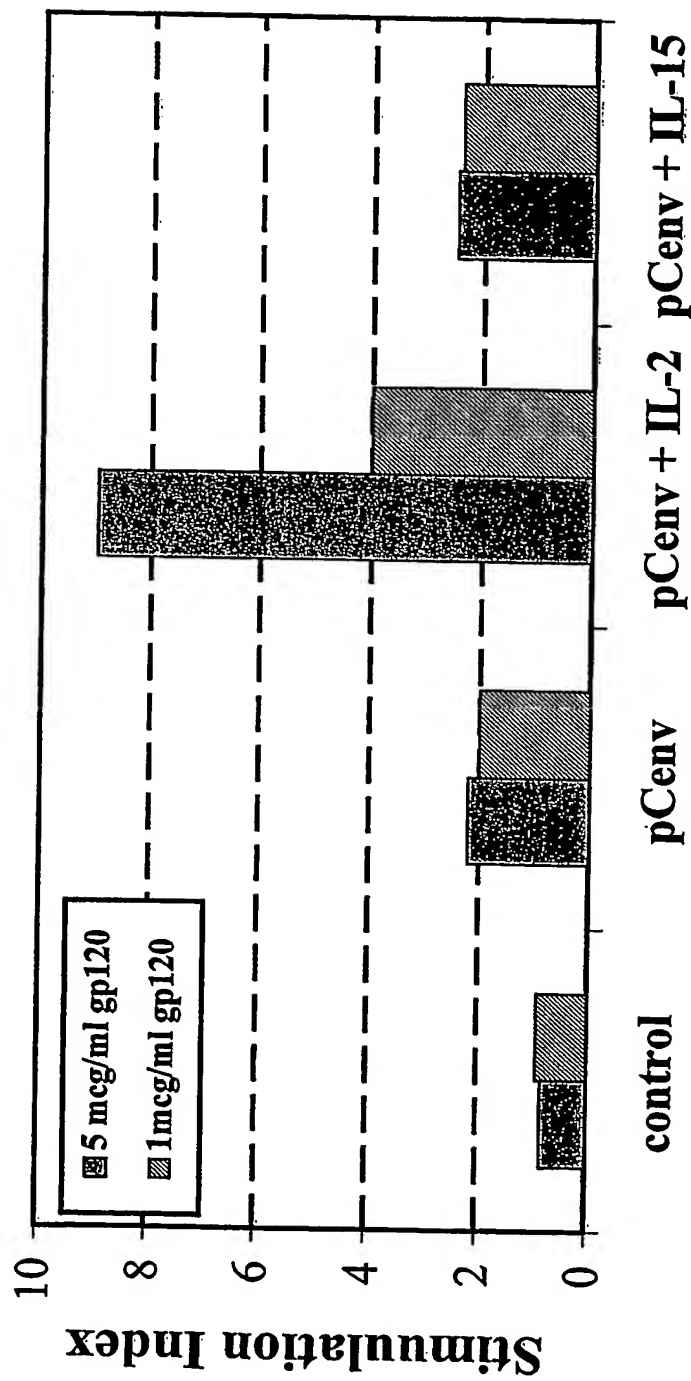


FIGURE 6

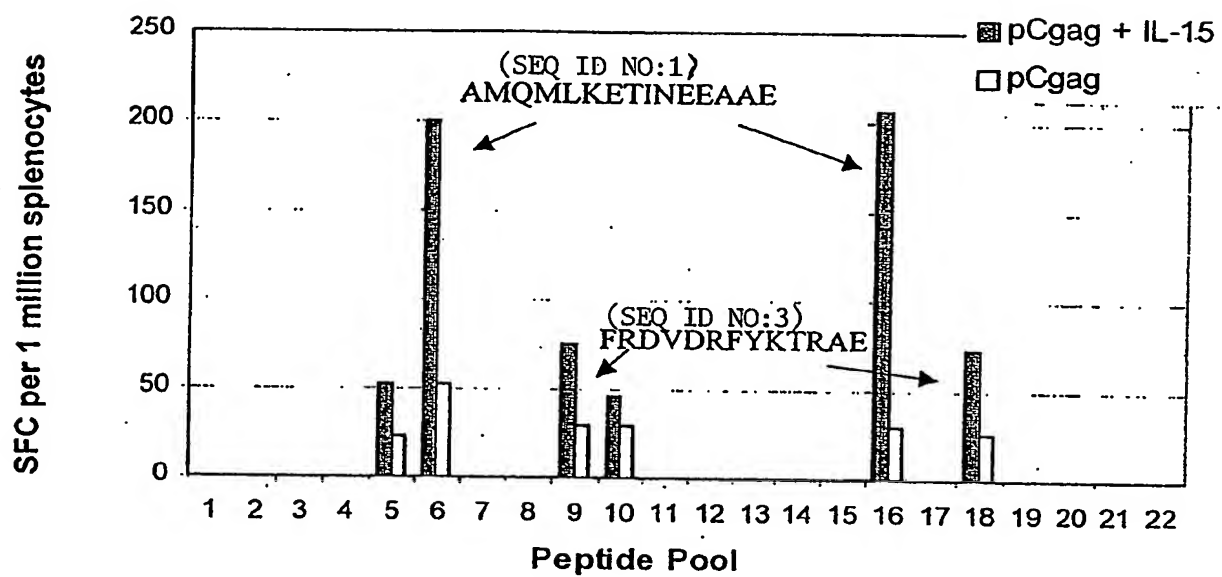
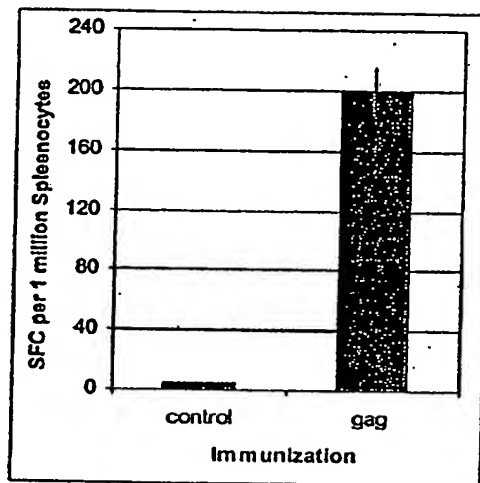
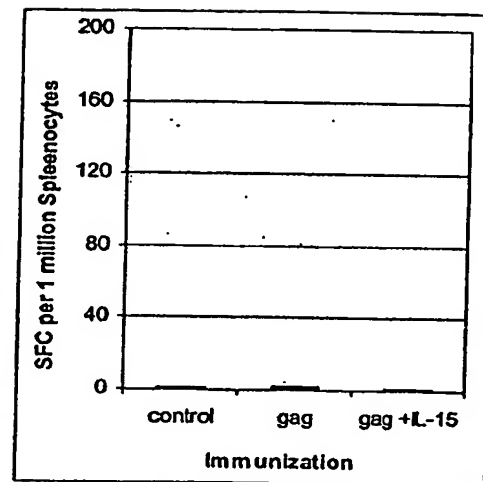


Figure 7

A



B



C

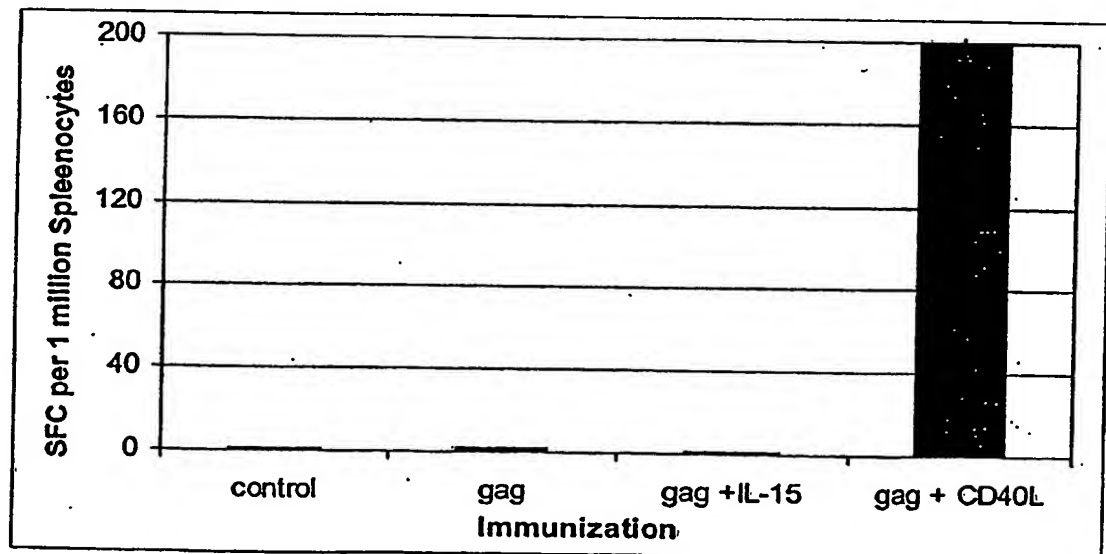


Figure 8

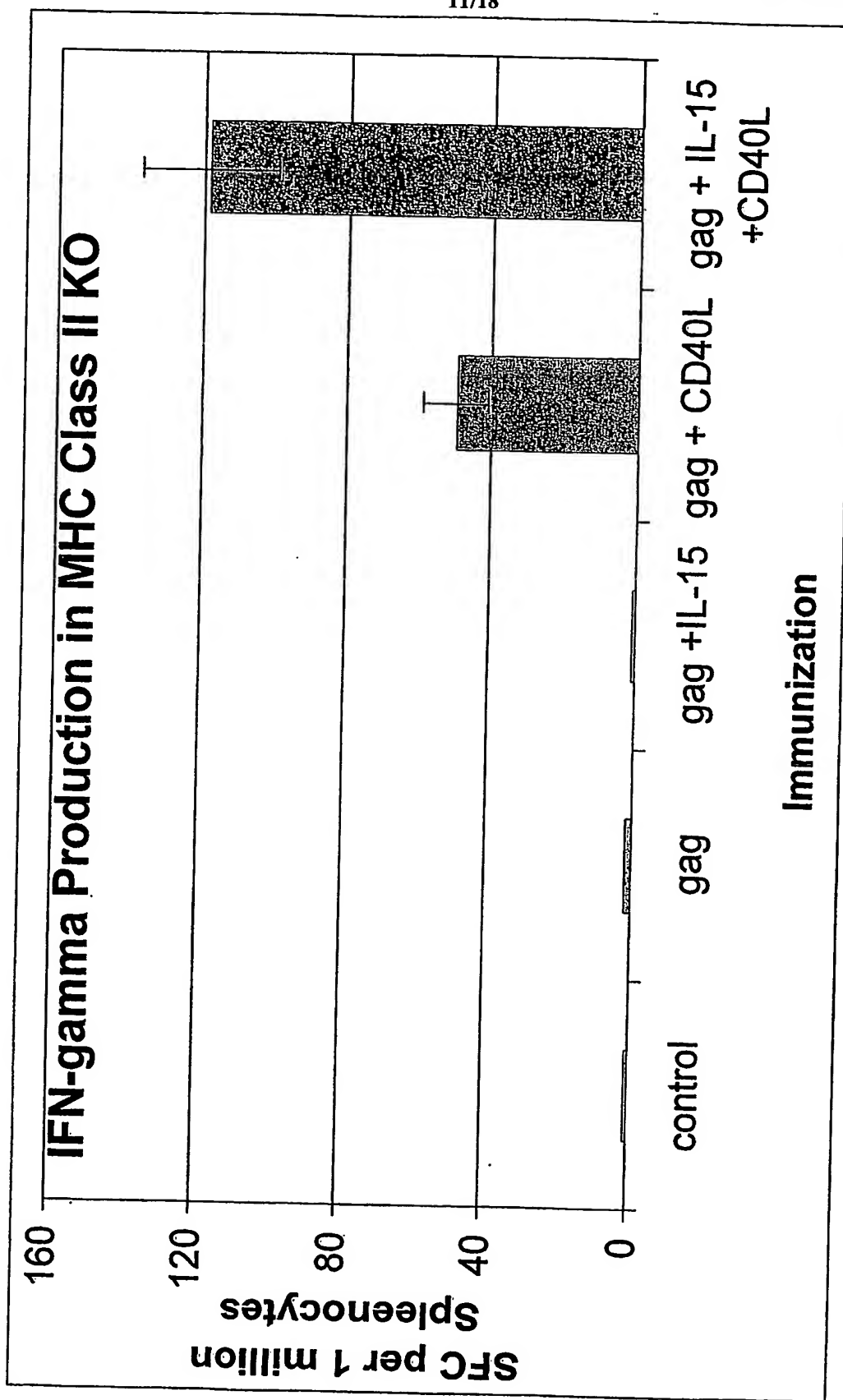


FIGURE 9

Strategy for Increasing Expression of IL-15 through Optimization of IL-15 DNA Constructs for Immunization

- ❖ Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15 (LSP)	sense (SEQ ID NO:4)	GCCGCCGTCGAC GCCGCCACCATGAGATTTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAAGTGTGATGAACATTGG
Macaque IL-15 (LSP)	sense (SEQ ID NO:4)	GCCGCCGTCGAC GCCGCCACCATGAGATTTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAAGTGTGATGAACATTGG
Human IL-15 (SSP)	sense (SEQ ID NO:6)	GCCGCCGCTACC GCCGCCACCATGGTATTGGGAACCAT
	antisense (SEQ ID NO:7)	ATCGGGGGGATGC TCAAGAAAGTGTGATGAACAT
Legend: Restriction Site, KOZAK, START, STOP CODON		

FIGURE 10

Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- ❖ Sense primers are designed to start after 48 aa ISP while antisense primer amplifies from stop site.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense (SEQ ID NO: 8)	GCCCCCGAATTG GCCGCCACCATGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGC-TACTAGAGTTTCATTCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGGCTGGAG TCAGCAAGTGTTCATGAACATTGG
Macaque IL-15-IgE	sense (SEQ ID NO: 8)	GCCCCCGAATTG GCCGCCACCATGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTTCATTCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGGCTGGAG TCAGCAAGTGTTCATGAACATTGG

Legend: Restriction Site, KOZAK, START, STOP CODON

FIGURE 11

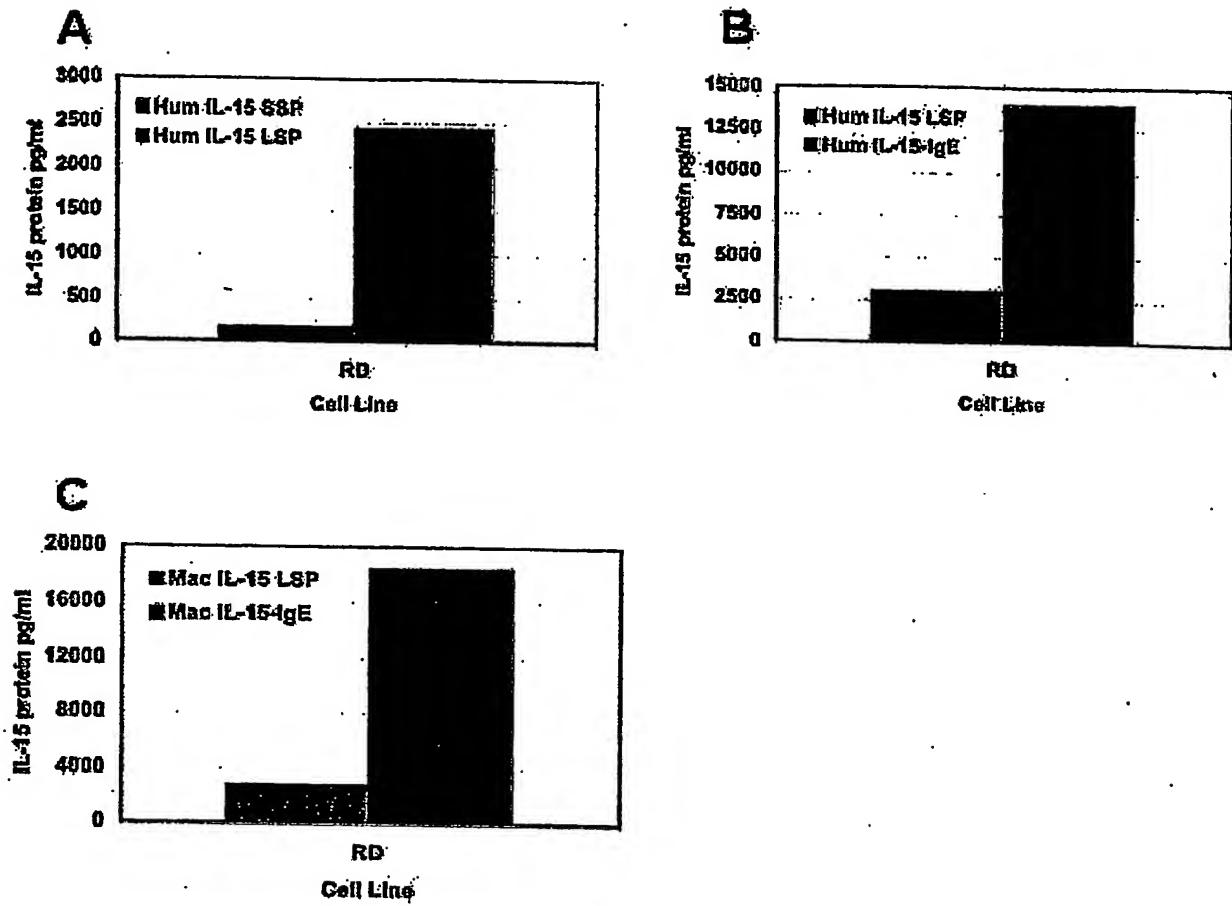


FIGURE 12

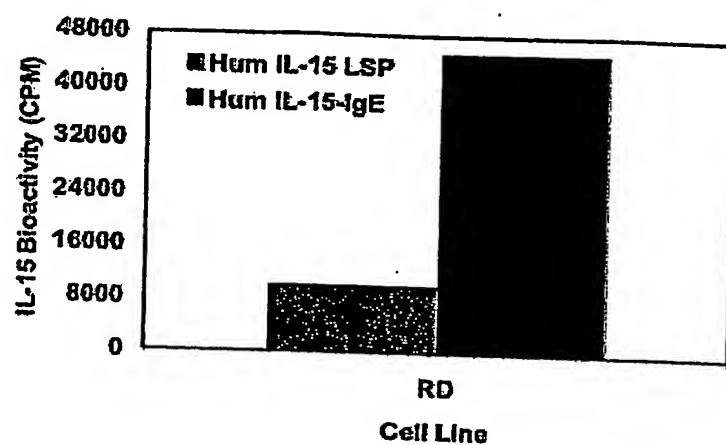
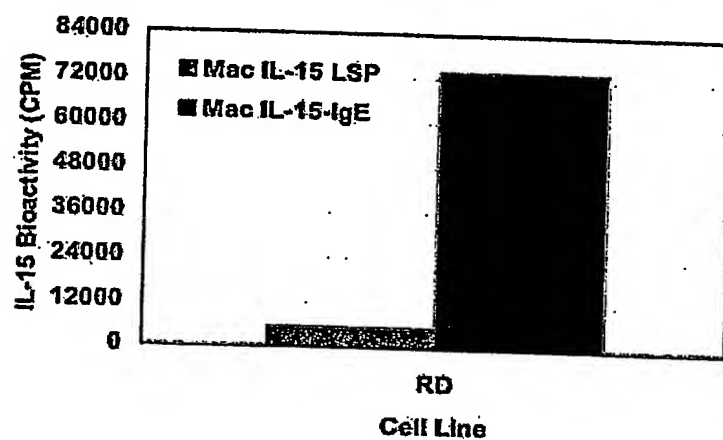
A**B**

FIGURE 13

Immunization Schedule

Immunization Groups:

Naïve

Vector Control

HIV-1 Gag

HIV-1 Gag/ IL15 constructs



*Combinations of 100 µg IL15 Constructs, 50 µg GAG,
Each injection, intramuscular*

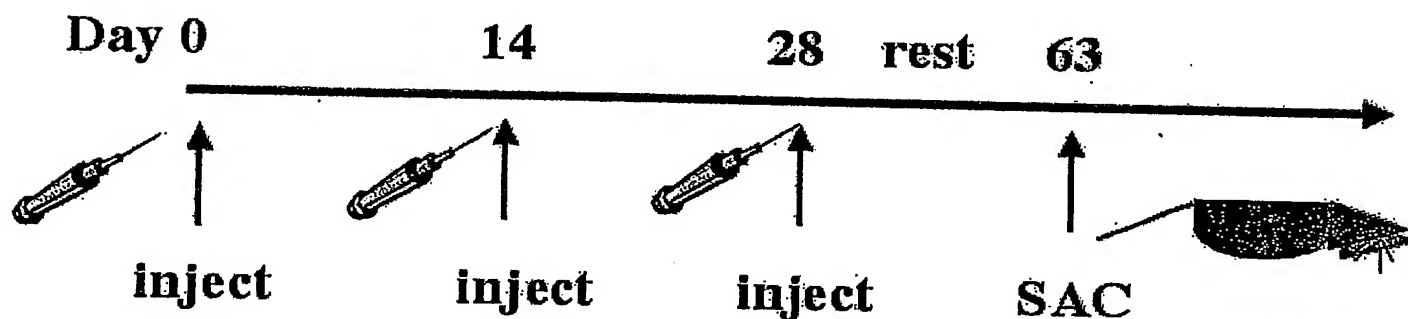


FIGURE 14

Restimulation of antigen-specific IFN- γ production 5 Weeks Following
the 3rd immunization of HIV-1 Gag in Balb/C mice
Effect of IL-15 Constructs

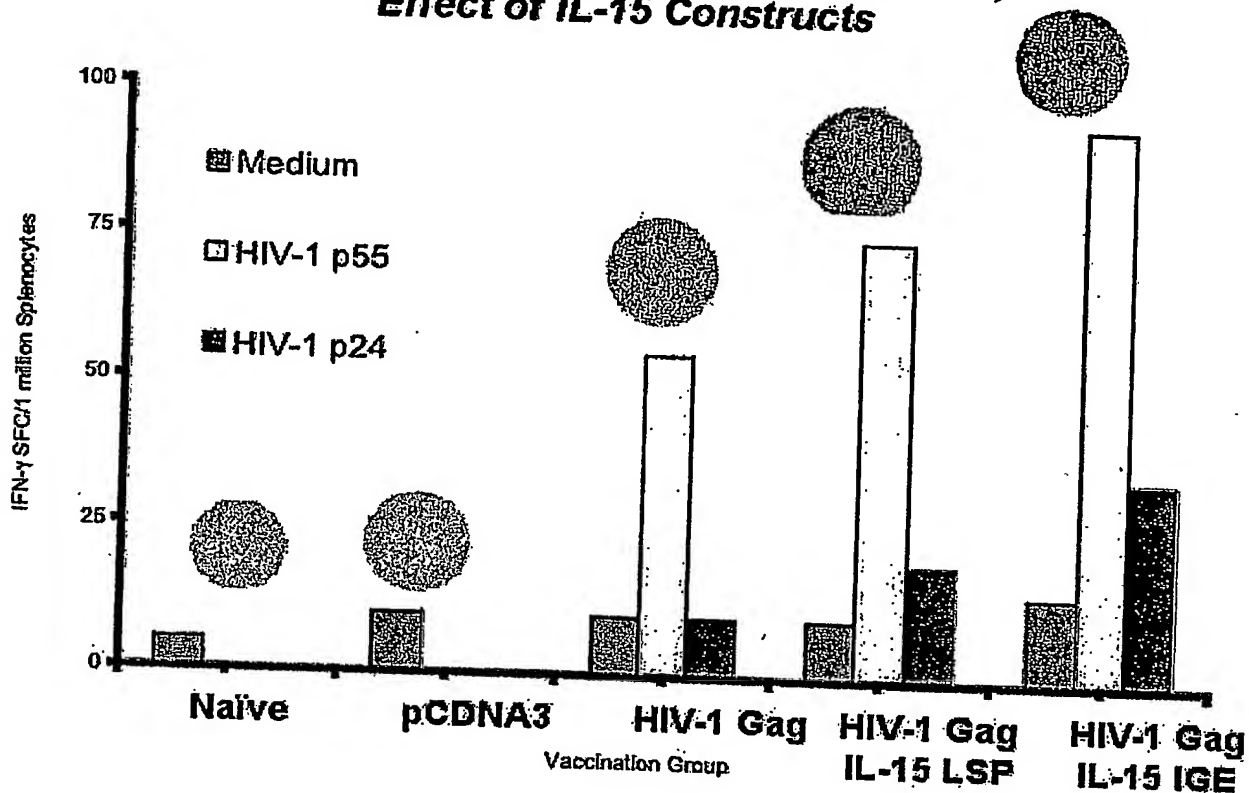


FIGURE 15

An Engineered IL-15 Plasmid Vaccine
(Kozak, AUG's removed, UTR's removed & other-30-
50X better expression)

Enhances CTL response in Vivo
Mice were immunized with HIV-1 gag expressing DNA

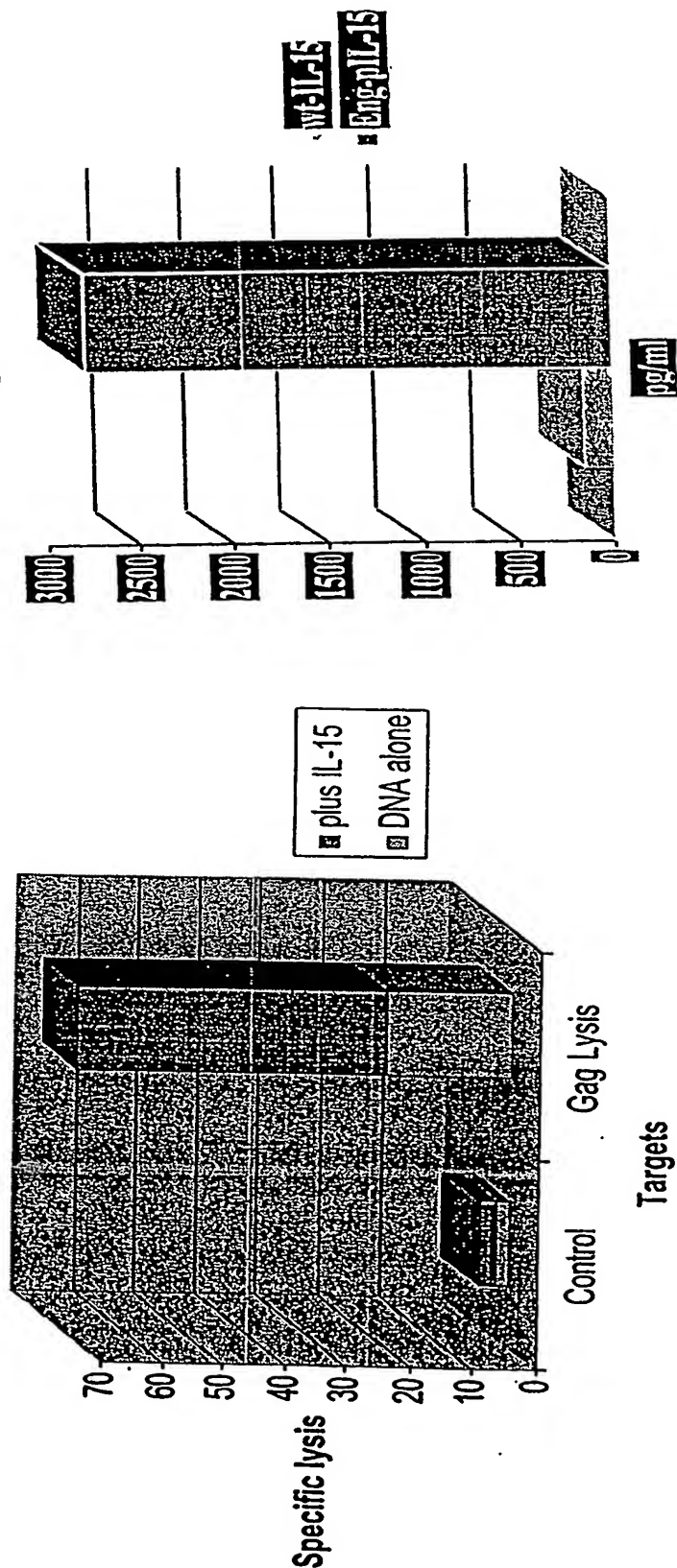


FIGURE 16

Grabstein et al. (1994) Science 264:965-968, Bamford et al., 1996) PNAS 93:2897-2902
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903

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